BIOTECHNOLOGY IN FOOD PROCESSING

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Foreword

Biotechnology 1945-1985

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Dedication

Dedicated to the memory of Edward L. Tatum (1909-1975)

The editors requested, or at least acquiesced in the suggestion, that I look back over the 40 years of my own active involvement in biotechnology research. My historical focus will be on three of the publications that underlay much of the theoretical insight and experimental methodology used in biotechnology today: Beadle and Tatum (1941); Avery, MacLeod and McCarty (1944) and Lederberg and Tatum (1946). They concern biochemical genetics *(Neurospora)*, DNA mediated transformation (pneumococcus) and conjugal gene exchange (*E. coli* K12), respectively. Think back to, or for most of you try to imagine, a time when genetic research had not yet reached *E. coli* K12 as an experimental object; when the very notion of genes in bacteria was problematical. Table 1 summarizes some of the milestones of physiological genetics before 1945.

Our discipline really does begin in the middle of the last century. We had Gregor J. Mendel with the first account of genes as segregating units in carefully designed crosses of garden plants. We had Friedrich Miescher with the first isolation of a material that we now know as nucleic acid. Within a few years we had the pure culture of microorganisms and their role in disease, putrefaction and fermentation clearly established. Mendel's work lay dormant for 35 years to be suddenly rediscovered by three groups of investigators (de Vries, Correns, and Tschermak) simultaneously in 1900: the beginning of modern genetics.

Within two years Archibald Garrod, an internist, was looking at a trait called alcaptonuria, distinguished by the blackening of urine. He studied the pedigrees and with Bateson's help inferred that this attribute followed the

recently rediscovered Mendel's Laws. Over the next decade he established several clear examples of "inborn errors of metabolism:" genetic blocks of a metabolic pathway. Garrod's work was only dimly recognized by mainstream genetics. It did appear in textbooks of biochemistry and medicine. It will come up again because some of the very concepts that Garrod used in his explanation of these diseases in man were to be redeveloped in the modern era of biochemical genetics.

Table 1. History of Ideas Leading to Crossing Bacteria, (*E. coli* K12)

	Genetics
1865	Mendel—first account of genes
1900	deVries, Correns, Tschermak—rediscovery
1902	Garrod—inborn errors of metabolism
1904	Blakeslee—*Mucor*
1928	Dodge—*Neurospora* life cycle
1941	Beadle & Tatum—*Neurospora* biochemical mutants
1942	F.J. Ryan—*Neurospora* biochemical mutants at Columbia U.
	Biochemistry
1865	Miescher—nucleic acids
1920	Levine—nucleotides
1926	Sumner—crystalline urease: protein
1930	Northrop—crystalline pepsin: protein
1935	Stanley—crystalline TMV: protein plus RNA
1944	Avery, MacLeod, McCarty—DNA has genetic activity in pneumococcus
	Microbiology
1676	van Leeuwenhoek—bacteria
1695	van Leeuwenhoek—protozoa copulating
1870's	Pasteur, Koch, Cohn—*Schizomycetes*
1928	Griffith—pneumococcus transformation
1930's	*Salmonella* serotypes
1943	Luria & Delbruck—population statistics of bacterial mutations
1944	USNH—malaria life cycle
1945	Dubos—"The Bacterial Cell" contra "Traditional" medical school teaching
1945-6	Lederberg (Columbia, Yale)—*Escherichia coli* K12

One early venture into microbial genetics did flourish briefly between 1900 and 1941. Albert Blakeslee wrote his doctoral thesis at Harvard University on the sexual cycle of the *Mucorales* in 1904. His work with a few species of *Mucor* and *Phycomyces* established a segregation of sex determining factors in these fungi, and he was obviously influenced, like Garrod, by the resurgence of Mendelism. However after he completed his doctoral work he could not get a job working on the genetics of fungi; it had no obvious agricultural or any other applied significance. Instead he was able to get work at the Agricultural Experiment Station in Connecticut and began his work in plant breeding and cytogenetics where he did make many important contributions. So fungal genetics lay fallow for several decades until other sources of interest in fungi, their life cycle and their application to genetics could be revived.

In 1928, B.O. Dodge dearly loved one of these fungi that had been originally isolated in Java growing on moldy peanuts, and then other species growing on bread—the red bread mold *Neurospora*. He found that *Neurospora* was uncommonly easy to handle and to carry through its complete sexual cycle. He worked out the segregation of sex in this particular organism and found that whereas the zygospores in *Phycomyces* do not germinate very efficiently, the ascospores in *Neurospora* do. It ended up being an ideal organism for Mendelian genetics.

George Beadle and Edward Tatum come into our story out of their efforts to discover genetic markers with which to probe the relationship of genes to development. Among the earliest mutants discovered in *Drosophila* were albino or other pigment anomalies in eye color. It was obvious that there was a chemical difference in white eyes versus red eyes, and some effort was made in the extraction of these pigments. Around 1935, Boris Ephrussi and George Beadle discovered diffusible substances which were accumulating in the larval body of some mutants and could restore the color of implants of eye primordia from other mutants; what we would now call a simple complementation test. They called this diffusible substance the V+ hormone. We would now call this "hormone" a metabolic intermediate in a blocked chemical pathway. Beadle then went to Stanford University in 1937 to continue these studies and advertised for a biochemist to help him in the conduct of this work. Tatum had done his doctoral work at the University of Wisconsin at Madison. Shortly thereafter with Harland Wood he was the first to show that propionic bacteria had a growth factor requirement for thiamine, a vitamin previously known to be important in the metabolism of mammals and of yeast. From that experience he became thoroughly imbued with a sense of comparative biochemistry; namely that many metabolic systems would be found to be very similar in a wide variety of organisms. He thus felt unrestrained in his choice of experimental material.

At Stanford University, this sense was soon to be demonstrated with a vengeance in his work on *Neurospora*. His immediate task was to isolate the blocked intermediate, the V+ hormone, out of the fruit flies. Isolating it by the gram was a formidable task, for they had few modern methods like chromotographic isolation. Then, at the very last minute, after having done all this backbreaking work, they were scooped by Butenandt. He had tested a substance that had been found in dog urine and believed to be an anabolite of tryptophane, namely kynurenine. It functioned in the bioassay test exactly like the elusive V+ hormone. That easy win just leapfrogged all over the various laborious isolations that Tatum had done throughout this time. This chastening experience motivated them to seek better experimental material for biochemical study. Guided in part by Tatum's prior background in microbial nutrition it occurred to Beadle that they could reverse their research strategy. Instead of painfully pursuing the biochemistry of the mutants one happened to find in

Drosophila, why not pick an organism where one could look for mutants blocked in specific nutritional pathways? In other words, by mutational block, create additional examples of what nature had provided in the biochemical requirements; e.g., for thiamine in the propionibacterium. It worked like a charm within a few months. The project involved irradiating *Neurospora*, crossing the irradiated spores to cultures of the opposite mating type, isolating single ascospore cultures and testing them for growth on basal medium. It took as few as 299 manual isolations of single spores, which is a couple of days' work once you get the hang of it, before they had a culture which had an induced requirement for pyridoxine. The growth requirement segregated in Mendelian fashion; and the modern era of experimental biochemical genetics had begun.

Another milestone appeared in 1928, a casual observation outside of any contemporary paradigm. Fred Griffith was working on the very practical problems of the serological classification of the organisms of pneumonia. He pondered what was different about type 1, type 2, type 3, and so on which were recognized by the serological reagents. His experiment, the speculative roots of which were not clearly explained in his published paper, was to put killed cells of one type into a mouse. He then inoculated the mouse with live cells of another type and came out with live cells of the first type; i.e., of the killed organisms. So he postulated a transforming principle that on some occasion would transfer an attribute from those dead bacterial cells to the ones that were still living. This work, published in the Journal of Hygiene, hardly reached the attention of geneticists. In any case there was no framework with which to understand its biological significance and no single geneticist in those days was working on bacteria as test organisms.

O.T. Avery, a biochemist and microbiologist at The Rockefeller Institute, was very much concerned with pneumonia for the same reasons as was Griffith. When Griffith's work was first announced he was quite skeptical. However, some of his postdoctoral associates were able to repeat Griffith's published observation. We certainly have to credit Avery and the leadership of The Rockefeller Institute for devoting a large part of the energy and resources of his laboratory from 1930 to 1944 to doing what he did best—the fractionation, isolation and characterization of chemical entities; the extraction of the material out of those killed pneumococcal cells that could cause the transformation of types. Much to Avery's astonishment, this material turned out not to be protein (which the theory of the time would have predicted) but DNA. It is perfectly correct to say that the DNA revolution began with this finding by Avery, MacLeod and McCarty: the first operational assay for the biological specificity of a preparation of DNA.

Let us jump now to 1945. Beadle and Tatum have published a series of papers on *Neurospora* out of Stanford University. Avery and his group have published their report that the transforming factor in the pneumococcus is DNA. I find myself, a medical student, working with Francis J. Ryan in the Department of Zoology at Columbia University. In fact I had begun working for him in my undergraduate sophomore year, 1942, when Ryan returned from his postdoctoral fellowship with Beadle and Tatum. He was one of the first people to bring *Neurospora* out of the Stanford Laboratory. Of course in the

department we were all full of the excitement of this new world of biochemical genetics.

It is hard to convey the meteoric impact of Avery's work. Well before it was formally published, we heard about it from Alfred Mirsky who traveled frequently between The Rockefeller Institute and Columbia University. The inspiration that this was going to be the key to what we would now call molecular biology was transparent to everybody in the department. I say this with such vehemence because others have denied that Avery's work was well understood. Perhaps it was not well understood in other quarters, but in a place that has the metabolism of knowledge that New York does, the atmosphere was really quite electrified. This had to be the opening of the door to a great new era, and we had to find every conceivable way to try to exploit this breakthrough.

There were problems on all sides; e.g., whether the transforming factor was pure DNA in the first place. At one level that didn't matter too much, for the course had already been clearly set and in short order it would be settled as to whether the gene was DNA or protein, or both, or something else. If the pneumococcal transformation factor was really a gene, a unit of Mendelian heredity, how could we verify that fact, when one knew nothing else of the genetics of bacteria?

The first effort was to try to transform *Neurospora* with DNA containing extracts. Ryan had taught me the technology of the auxotrophic mutants that would not grow in a simple synthetic medium without some supplement. So it was not difficult to imagine an experimental design that would greatly facilitate looking for transformation in this organism; namely to seek a few transformed cells (prototrophs) that would grow without the supplement. If you could get DNA transfer in *Neurospora*, one could answer all the questions that separated pneumococcus from *Drosophila*. Well it didn't work! But I won't take too much discredit for that, for it is only in the last three or four years that systems have been developed to enable *Neurospora* to be transformed, and they do require very arcane handling of the organism to get DNA into it.

That was discouraging, but at least it had laid out the experimental methodology by which one could efficiently look for genetic transfer in a microorganism. So to ring the changes, if we can't transform *Neurospora* maybe we can develop a broader genetics of easily handled bacteria like *E. coli*.

Tatum, having a very strong bacteriological background himself, had started to generalize on the study of metabolic pathways from *Neurospora* to *E. coli*, and in late 1944 he published a report on auxotrophic mutants of the same variety as had been found in *Neurospora*. This was an analogy between *E. coli* and *Neurospora* that would at least encourage one to look further into attempts to dissect its genetic structure. But there were no known crosses, no transformations, no other methods of dealing with the relationships of genes to one another, and certainly no way to identify them as DNA. Based on these premises, there were two further lines of experiment. One that I did in a rather desultory way was to try to transform *E. coli*, but it was to take many years to concoct the witches' brew that makes it possible (calcium treatment and so forth).

Another possibility emerged out of the need, this desperate need, to do

something genetically significant with *E. coli* in order to justify the Avery experiment and its generalization to genetics. If you could show that bacteria could be crossed, a bridge between Avery's experiment and the rest of the world of genetics would be completed. So that led to a reexamination of whether *E. coli* could be crossed.

The experimental design recorded in my notebook dated July 15, 1945 was "Diplophase in bacteria can be selected for by using two different mutant strains of *E. coli* and growing them in continuously renewed minimal medium." In practice it sufficed to plate washed cells from a mixed culture onto minimal agar.

Francis Ryan encouraged me to continue these experiments with Ed Tatum. He had just left Stanford (where biology departments in those days did not have that much use for biochemists) and he was to start a new department of microbiology at Yale University. He had then just published a second research paper which explained that he had taken some of the single step mutants in *E. coli*, mentioned before, and subjected them to a second round of radiation to yield double mutants.

These double mutants were exactly what I had been looking for to prevent "adaptation or reverse mutation." The design was: if there were two genetic blocks in each culture, then the statistical probability of both undergoing reverse mutation in a single selection cycle would be reduced to a negligible quantity. We were getting reversion rates of 10^{-6} or 10^{-7} per cell—enough to interfere with the selection experiment. One could expect double mutants to be occurring at 10^{-12} or 10^{-14} and that is a sufficiently small number to give acceptable blanks.

Tatum very graciously accepted my proposal and invited me to join him in New Haven. I arrived in New Haven in March 1946, started assembling the mutants, made a bunch more, and did the first actual crossing experiments on the 2nd of June. Soon I had done about a dozen of them and was therefore quite convinced that the experiment would work and be reproducible. The Cold Spring Harbor Symposium in 1946 was coming up in early July. We had no plans to present the material after just one month of experimentation; but this was the first international conference of microbial genetics. Everyone in the world was there and almost everyone was bewailing the lack of a sexual process in bacteria. So we decided to present the first results even with that very limited number of experiments.

We were fortunate to be able to get the first confrontation in front of nearly every expert in the world all at once, under the discipline of their mutual critical outlook.

Things certainly have changed from then to now. A comparison of the earlier linkage map published for *E. coli* to a contemporary one shows a fantastic increase in the number of gene loci. Back then we knew nothing about F^+ or F^- (male and female). Presently there are well over a thousand loci mapped in *E. coli*, close to a third of the total expected to be there.

May I now take up a question that has dogged me for many, many years? Why did it take until 1946 for successful crossing in bacteria? The experiment I've described to you is so simple that many high school students have done it. Many of the concepts needed to conduct it were available in the early 1900's.

Why wasn't it done then? Why did it take 40 years?

To answer this question, let us review some older history. Microbiology began with the first microscopic visualization of microbes at the hands and eyes of Leeuwenhoek in 1676. He continued his observations and in 1695 he reported on a wide variety of animalcules, larger than the tiny dancing dots, the bacteria. These larger animals were the protozoa. He gave an eloquent description of the copulation of protozoa that made it clear that they indeed had a sexual process. That protozoa were sexual while bacteria were not was then an attribution of the scale of nature that was firmly engrained with the very initiation of microbiology. This was reaffirmed with the development of pure culture methodology by Koch and Cohn. This concept of bacteria as organisms that breed true to type and do not divide by any other process than fission became crystalized in 1875 in the first formal taxonomy of bacteria. Ferdinand Cohn called them the *Schizomycetes*, the fission fungi. The myth of bacterial asexuality was thus engrained in the very class names of the organisms in question. The compartmentalization of teaching, of thought, reflected in the separation of microbiology as a medical specialty with little interest in the fundamental biology of these organisms, the contrary with respect to genetics, is at least part of the explanation for bacterial genetics having a delayed start.

Today, the intersection of applied research with fundamental study is being pursued on a broad interdisciplinary foundation. As I look back over the most important and revolutionary advances in science, I observe that many of them have come from the intersection of the world of practice (medical practice, engineering needs, biotechnological applications, natural history) with the cutting edge of existing knowledge. In a firmly established discipline the canon of experimental design is to narrow down the relevant variables and to control all the appropriate inputs to allow a reproducible and controllable result. That is just fine as long as your theoretical outlook suffices to embrace all the relevant variables. In that world of practice one can no longer choose the constraints for experimental convenience. The real world invariably intrudes operational variables you hadn't thought about, had no way of controlling, and many new discoveries have come from that sphere. For example, I am confident that efforts to optimize biotechnical organisms for hyperproduction will uncover new principles of gene regulation. In this, and in many other ways, I am confident that research with applied orientation, such as is being celebrated at this symposium, will also surely nourish the most revolutionary findings when one looks beyond the most proximal practical goals. In complementary fashion, the most exciting advances in technology have resulted from pure science in totally unrelated fields. When Ed Tatum was pondering where to begin his definitive work after his postdoctoral fellowship, his mentors urged him to go into the microbiology of butter. He could hardly have made a more important contribution to food processing biotechnology than what he did, beginning with the eye pigments of the fruit fly.

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BIBLIOGRAPHY

1. Beadle, G.W. and Tatum, E.L. Genetic control of biochemical reactions in *Neurospora*. Proc. Nat. Acad. Sci. 27:499–506 (1941).
2. Avery, O.T., MacLeod, C.M., and McCarty, M. Studies on the chemical nature of the substance inducing transformation of pneumococcal types. J. Exptl. Med. 79:137–158 (1944).
3. Lederberg, J. and Tatum, E.L. Gene recombinations in *Escherichia coli*. Nature 158:558 (1946).